The Systematic, Adaptive and Physiological Significance of Proteolytic Enzyme Distribution in Bivalves

BY

ROBERT G. B. REID

Department of Biology, University of Victoria, Victoria, British Columbia, Canada

INTRODUCTION

THE DISTRIBUTION OF digestive enzymes in animals is believed to be adaptive, and related to the constituents of the diet (YONGE, 1937). Most bivalves have a low protein diet. Consequently, high levels of proteolysis would not be expected, and there has been relatively little interest in protein digestion in this class of molluscs. YONGE (1926) was satisfied that in *Ostrea edulis* Linnaeus, 1758 protein digestion was an intracellular process in the amoebocytes and digestive cells of the digestive diverticula, and he contended that gastric protein digestion would be incompatible with proteinaceous crystalline styles (YONGE, 1930). Therefore some controversy was aroused when MANSOUR (1946) and MANSOUR & ZAKI (1946) argued that the digestive diverticula of bivalves were secretory and that animal food could be digested in the stomach. However, the low levels of protein digestion in the stomachs of *Tridacna elongata* Lamarck, 1819, and *Pinctada vulgaris* Linnaeus, 1758 detected by MANSOUR-BEK (1946) seemed insufficient to justify Mansour's claim. BALLANTINE & MORTON (1956) noted the presence of gastric proteinases in *Lasaea rubra* (Montagu, 1803), and suggested that they were released from "excretory spheres" from the digestive diverticula. OWEN (1956) examined *Nucula sulcata* Bronn, 1831 and found significant gastric protein digestion, but no diverticular activity.

Differences in the distribution of proteolytic enzymes of two herbivorous, suspension-feeding bivalves, *Lima hians* (Gmelin, 1791) and *Mya arenaria* Linnaeus, 1758 indicated an adaptive relationship between enzyme distribution and gastric morphology (REID, 1966). This led to an investigation of animals belonging to different taxa proposed by PURCHON (1963) on the basis of stomach morphology. This study (REID, 1968) provided some support for the original hypothesis concerning the relationship between stomach form and digestion. KOZLOVSKAYA & VASKOVSKY (1970) surveyed a variety of marine invertebrates, including 14 species of bivalves, for alkaline proteases. For the bivalves only the digestive diverticula were assayed. A range of results from zero activity to low activity were obtained for the bivalves, very low in comparison to carnivorous invertebrates. Since the survey dealt only with intracellular conditions it shed no light on the relationship between gastric morphology and digestion. However, a study of 7 species of *Macoma* (Tellinacea) by REID & RAUCHERT (1972) indicated that the suggested relationship between gastric morphology and protein digestion might be spurious. Within the genus *Macoma* a range of proteolytic levels was noted, though all the species have similar stomach structures. This problem was compounded when B. MORTON (1970, 1973) proposed that bivalves undergo rhythmic and possibly endogenous cycles of ingestion, secretion, digestion and absorption. Such cycles might involve proteases, and thus account for differences observed within the genus *Macoma*.

In carnivorous septibranchs there is high proteolytic activity which is related to the high-protein animal food (REID, 1977).

SYNOPSIS OF PREVIOUS OBSERVATIONS

To aid the comprehension of differences and similarities in protein digestion in bivalves, some of the relevant data are summarized below. The animals are grouped by family and gastric morphological type (PURCHON, 1956, 1957, 1958, 1960). Data are given in the following sequence: habit; level of gastric proteolysis; gastric enzymes if known; pH optima of gastric juice level of diverticular proteolysis; pH optima of diverticular extracts; diverticular enzymes if known. The stomach and the digestive diverticula are the main sites of protein digestion (REID, 1966). Gastric proteolytic activity is expressed in units per mL of stomach fluid and diverticular activity in units per mg fat-free dry weight of tissue. These are spectrophotometric units, each equivalent to an absorbance change of 0.001 per minute at 280 nm at 37° C. Absorbance increase is due to the release of amino acids as a result of proteolysis. Free amino acids present in extracts before enzyme assay are measured and deducted from final assay results. Soluble proteins which would also affect absorbance readings are removed by trichloracetic acid precipitation. Fuller technical details may be found in accounts by Reid (1966) and Reid & Rauchert (1970, 1972, 1976). In the following synopsis "low" gastric activity signifies 0 - 100 units per mL; "intermediate" gastric activity signifies 100 - 300 units per mL; "high" gastric activity signifies more than 300 units per mL. "Low" diverticular activity signifies 0 - 50 units per mg; "intermediate" diverticular activity signifies 50 - 150 units per mg; "high" diverticular activity signifies more than 150 units per mg.

Family Nuculidae, Stomach Type I

Acila castrensis (Hinds, 1843): deposit-feeder; intermediate gastric proteolysis; peaks at pH 3, 6, 7.5; diverticular condition unknown. (unpublished observations of author)

Family Nuculanidae, Stomach Type I

Yoldia thraciaeformis Storer, 1838: deposit-feeder, intermediate gastric proteolysis; peaks at pH 3.5, 5.5, 7.5; diverticular condition unknown (Reid, 1977).

Family Cuspidariidae, Stomach Type II

Cardiomya planetica Dall, 1908: carnivorous; high gastric proteolysis; peaks at pH 3, 6, 7.5; enzymes cathepsin B and trypsin; diverticular condition unknown (Reid, 1977).

Family Mytilidae, Stomach Type III

Mytilus californianus Conrad, 1837; suspension-feeder; low gastric proteolysis; peaks at pH 6, 7.5; trypsin; low diverticular proteolysis; peaks at pH 3, 6, 8 trypsin (unpublished observations of author).

Family Ostreidae, Stomach Type III

Crassostrea gigas (Thunberg, 1793): suspension-feeder; low gastric proteolysis; intermediate diverticular proteolysis; peaks at pH 3, 6, 7.5 (unpublished observations of author).

Family Pectinidae, Stomach Type IV

Chlamys hericius (Gould, 1850): suspension-feeder; low gastric proteolysis; trypsin; low diverticular proteolysis; peaks at pH 2.5, 6, 7.5; chymotrypsin (Reid & Rauchert, 1970).

Family Limidae, Stomach Type IV

Lima hians (Gmelin, 1791): suspension-feeder; low gastric and diverticular proteolysis; diverticular peaks at pH 5.5 and 8 (Reid, 1966).

Family Unionidae, Stomach Type IV

Anodonta kennerlyi Lea, 1860: suspension-feeder; tryptic gastric and chymotryptic diverticular enzymes (unpublished observations of author).

Family Veneridae, Stomach Type V

Saxidomus giganteus Deshayes, 1839: suspension-feeder; low gastric proteolysis; peak at pH 5.2; low to intermediate diverticular activity; peaks at pH 3, 6, 8; trypsin (Reid, 1977 and unpublished observations).

Family Mactridae, Stomach Type V

Tresus capax (Gould, 1850): suspension-feeder; low gastric proteolysis; peaks at pH 2, 5, 8; gastric cathepsin B and trypsin; intermediate diverticular proteolysis; peaks at pH 2.5, 6, 8; diverticular cathepsin B and D and chymotrypsin (Reid & Rauchert, 1976).

Family Tellinidae, Stomach Type V

Macoma secta (Conrad, 1837): large particle deposit-feeder; intermediate gastric proteolysis; peak at pH 6; gastric trypsin; intermediate diverticular proteolysis; peaks at 2.5, 5.5, 8; diverticular chymotrypsin (Reid & Rauchert, 1972).

Macoma inquinata (Deshayes, 1854), suspension-feeder; intermediate gastric proteolysis; peaks at pH 5.5, 7; intermediate to high diverticular proteolysis; peaks at pH 3, 5, 7.5 (Reid & Rauchert, 1972).

Macoma lipara Dall, 1916: deposit-feeder; low gastric proteolysis; peaks at pH 7; low to intermediate diverticular proteolysis (Reid & Rauchert, 1972).

Family Myidae, Stomach Type V

Mya arenaria Linnaeus, 1758; suspension-feeder; intermediate gastric proteolysis; peaks at pH 5.5, 7.2; cathepsin B and trypsin; intermediate diverticular activity; peaks at pH 2.5, 3.5, 5.5, 8; diverticular cathepsin B, trypsin (Rosén, 1949; Kozlovskaya & Vaskovsky, 1970; Reid, 1966 and unpublished observations).

Before discussion of these data some qualifications must be noted: in some of the earlier works only qualitative observations were made. In most cases, enzyme assays are incomplete; consequently, the identification of certain enzymes in some examples does not imply that those enzymes are absent from other animals investigated. The studies on *Chlamys hericius* and *Tresus capax* indicated the presence of carboxypeptidases and aminopeptidase in both the acid and alkaline pH range, as well as possible cathepsin A and cathepsin C activity. These exopeptidases are probably of universal distribution. The results of Kozlovskaya & Vaskovsky (1970) are largely excluded from the above due to the different expressions of unit activity employed by them. It is of note that 7 of their 14 bivalve species showed no alkaline endopeptidase activity. *Mya arenaria* showed the most activity. In addition to the data summarized above, I have made observations concerning variations in proteolytic activity within species. In *Macoma secta*, samples taken in May for 2 consecutive years were found to have significantly different proteolytic levels, and gastric protein digestion in *Macoma inquinata* was found to vary significantly (Reid & Rauchert, 1972). Further observations on variations in *Tresus capax* are presented in the following report.

PRELIMINARY OBSERVATIONS on the DIGESTIVE PHYSIOLOGY of *Tresus capax* (Gould, 1850) in RELATION to TIDAL RHYTHMS

As noted above in the introduction, considerable interest has been generated in rhythmic physiological activity in bivalve alimentary tracts, by the work of Morton (1970, 1973).

Gastric protein digestion in *Macoma inquinata* was found to vary significantly, and these variations were attributed to feeding behaviour, relative to tidal conditions (Reid & Rauchert, 1972). Proteolytic levels and the relative activities of particular proteases would be direct indicators of rhythmicity. Accordingly a pilot study of *Tresus capax* was carried out, to investigate gastric volume, gastric pH and proteolytic activity, and digestive diverticular activity.

Tresus capax was chosen for its large size and because its proteolytic enzymes are better characterized than those of other bivalves (Reid & Rauchert, 1976). Specimens were obtained from the intertidal region at Cherry Point, in the vicinity of Satellite Channel, British Columbia. This is a locality with a mainly diurnal tide; *i. e.*, where at the spring tidal period the animals in their natural habitat are uncovered once in 24 hours. Specimens of similar shell size were placed in wire baskets and reburied in their natural habitat. The baskets were buoyed, so that they could be collected by boat at high water. After a 48 acclimatization period, during which it was verified that animals were able to use their siphons effectively, sampling began, was carried out at 3 hour intervals for 24 hours. During the 24 hours of sampling the experimental animals were exposed by the tide for 6 hours and submerged for 18 hours.

Gastric pH in the samples was found to range from pH 6.2 to 7.2. Exposed animals were in the lower part of the range, submerged animals in the higher part of the range. Gastric volume was lowest (1 mL) in exposed animals, and highest (up to 8 mL) in animals which had been submerged for 8 hours or more. Gastric proteolysis was low, but constant in all samples. Diverticular proteolysis appeared to be low in exposed animals and intermediate in submerged animals. Sample sizes were too small to provide statistically reliable conclusions beyond the above. There did not appear to be any drop in gastric volume and pH during the 18 hour submergence, which might have corresponded with the minor ebb, which did not expose the specimens. These observations are discussed under the heading "Protein digestion and rhythmic physiological events in bivalves" in the following general discussion.

DISCUSSION

General Features of Protein Digestion in Bivalves

In spite of the cursory nature of some of the observations reviewed above, it may be concluded that all bivalves are capable of digesting protein. Though comprehensive enzymological studies are few it may be inferred that most bivalves have a complement of most of the proteases which are found throughout the animal kingdom. Particular categories of proteases cannot be indentified with particular habits, gastric morphology, nor systematic status. In general, the pH optima of gastric enzymes correspond with the pH of the stomach, *i. e.*, in the pH 6 range (Morton, 1970; Mathers, 1973; Reid, 1977). In the digestive diverticula low pH (2 - 3.5), intermediate pH (4 - 6.5) and high pH (7 - 8) activity peaks occur universally. In *Tresus capax* the enzymes cathepsin D, cathepsin B and chymotrypsin are active in the low, intermediate and high pH regions respectively. This may hold true for other bivalves.

Protein Digestion and Gastric Morphology

It might be generalized that the stomach types such as I and II, which are structurally the simplest, are the ones which have the highest levels of proteolytic activity (*e. g.*, *Cardiomya, Yoldia*). It does not follow that there is an adaptive relationship, since it has been shown that there are significant differences in proteolytic levels within the type V group. The simplicity of the type I and II stomachs is probably more directly related to the triturative process than to the enzymatic process. No definitive adaptive relationship between protein digestion and gastric morphology can be stated at present.

Protein Digestion and Habit

The suspension-feeding bivalves which have been studied exhibit a range of proteolytic activity from low to intermediate in terms of the present study. The question of what constitutes the diet of suspension- and deposit-feeders has been discussed by Pohlo (1969) and Reid (1971). The protein content of the phytoplankton food of suspension feeders is variable, but does not exceed 30% by dry weight (Strickland, 1965). It is generally believed that deposit-feeders depend largely on the microorganisms associated with the detrital and inorganic particulate material of their diet (Newell, 1965). That the protein content of the food of deposit-feeders would warrant different levels of proteolytic enzymes from those found in suspension-feeders is debatable. However, it may be that gastric proteinases in some deposit-feeders play a significant role in separating potential food particles from substrate particles which have no food value, prior to endocytosis, as was suggested for *Macoma secta* (Reid & Rauchert, 1972). While this may also be the case for the deposit-feeding protobranchs it does not apply to other deposit-feeding members of the genus *Macoma*.

Only in the carnivorous septibranchs is there a positive relationship between diet and protein digestion. The family Cuspidariidae is the only group so far investigated which shows high levels of proteolysis. The diet consists of small copepods, ostracods, chaetognaths, polychaetes, etc. (Pelseneer, 1891; Yonge, 1928; Knudsen, 1970; Bernard, 1974), and the food is taken live (Reid & Reid, 1974). The major gastric proteolytic enzymes are trypsin and cathepsin B (Reid, 1977); *i. e.*, the same enzymes which are found in traces of the stomach of other bivalves. Thus, only a minor enzymological adaptation is involved, namely an increase in the synthesis and secretion rates, achievable by a simple gene duplication. In *Cardiomya* and *Cuspidaria* species the crystalline style is truncated, suggesting some attack by the gastric enzymes.

The Systematic Significance of Proteolytic Enzymes

There are a number of ways in which research into proteolytic enzymes might reveal their systematic significance. The first is the study of the primary structure of proteases of a given category. Although this would provide useful phylogenetic information about the bivalves and their affinities with other molluscs this expensive and time-consuming technique has not been applied. The second approach is the comparative study of protease zymograms; *i. e.*, the visualized patterns of electrophoretically separated enzyme extracts. The applicability of electrophoretic data to zoological systematics has been evaluated by Avise (1974). While a number of enzyme categories have been tested for their genetic or general systematic significance in bivalves (*e. g.*, Koehn & Mitton, 1972), and some of these studies have included categories of proteolytic enzyme (Levinton, 1975; Levinton & Fundiller, 1975) the information to date is insufficient to suggest clear systematic relationships. As Avise (*op. cit.*) points out, there are two schools of thought concerning the evolutionary significance of allozymes: the first is the selectionist view that each molecule has been tested by natural selection and is of positive value to the organism or population; the other view being the neutralist one which claims that some allozymatic variations have no particular superiority to others, but, since they are no less valuable, they remain as neutral mutations. Malacologists generally take the traditional selectionist view. However, if there is any truth in the neutralist view, then there is a possibility that some enzymological phenomena have no adaptive significance, but nevertheless have systematic significance. For example, in some members of the Pectinacea there is relatively high activity in the electrophoretically fast-running chymotrypsin. This might be a systematic phenomenon which lacks adaptive significance.

Protein Digestion and Rhythmic Physiological Events in Bivalves

J. E. Morton (1956) outlined phases of alimentary activity in *Lasaea rubra*. These were absorptive, intracellular digestive and excretory phases. The release of gastric proteinases was believed to be part of the excretory phase. B. S. Morton (1970) observed similar phases in *Cardium edule*, and inferred that these were digestive rhythms which correspond to tidal rhythms. He proposed that such rhythmic digestive phenomena were universal among bivalves, even those unaffected by the direct influence of the tide (Morton, 1973). Langton & Gabbott (1974) noted rhythmic changes in style pH, style protein, and amylase activity in *Ostrea edulis*, and concluded that these changes were modulated by the tide. However, they noted that the rhythms were lost following a period of continuous immersion. The pertinent literature has been more extensively reviewed by Owen (1974).

In my study of *Tresus capax* it was observed that gastric volume was at its lowest while animals were exposed and highest about 8 hours after submergence by the rising tide. Gastric pH was lowest in exposed animals and highest in submerged animals. The minor ebb, which did not expose the animals, had no obvious physiological effect.

It is particularly significant that the gastric proteolytic levels remained constant. This indicates an active release of enzymes as the animal feeds and the gastric volume increases. Otherwise the proteolytic activity would decrease with dilution.

The gastric pH changes are similar to those observed by Langton & Gabbott (1974) for oysters. The sequence of events outlined above may accord with Morton's hypotheses concerning rhythmic digestive phenomena in bivalves (Morton, 1973). However, the question of whether these events are endogenous, exogenous and modulated by the tide, or possibly synchronized by experimental artifact, remains open.

The Evolution of Protein Digestion in Bivalves

The range of categories of proteolytic enzymes present in bivalves is no different from that found in other invertebrates. In animals which have been closely examined most of the known invertebrate proteases have been found. The evolution of the endopeptidases is known to have been highly conservative in biochemical terms, and the view that animals, as they undergo adaptive radiation, encounter new diets and consequently evolve "new" digestive enzymes is fallacious with regard to the proteases. The range of peptide groupings in the most primitive protein substrate is the same as in the most highly evolved protein. Consequently, in the course of evolution, the need for a specifically novel category of protease is rarely necessary, though changes in the physiological environment might favour variants of pre-existing categories of enzyme.

While qualitative changes have not occurred in enzyme category, some innovative uses have been made by the bivalves of pre-existing enzymes, particularly in the use of cathepsin B for digestive purposes. In most organisms the lysosomes have a cathepsin complement responsible for autolysis, autophagy and general protein turnover. As indicated by seasonal changes in the relative levels of cathepsin in *Tresus capax* (Reid & Rauchert, 1976), cathepsin B has been turned to a digestive role and in the carnivorous septibranchs is employed for extracellular gastric digestion. A large quantitative change has been made by the septibranchs in response to their high protein diet. If the septibranchs were regarded as a primitive group of bivalves, and considering the protobranch condition, it might be concluded that the relatively high proteolytic capacity of the stomach is characteristic of primitive bivalves. However, considering the relative simplicity of the quantitative evolutionary step and the absence of qualitative differences it is impossible to justify such a conclusion, and, in general, in the absence of information on primary structure, proteases and the process of protein digestion are poor indicators of the direction of bivalve evolution.

SUMMARY

1. There are no systematically significant qualitative differences in the distribution of proteolytic enzymes in bivalves. Members of the Pectinacea have prominent, electrophoretically fast-running chymotryptic enzymes which may be of quantitative systematic significance. Electrophoretic zymograms of proteases may prove to be useful in determining systematic relationships.
2. No definitive adaptive relationships can be traced between protein digestion and gastric morphology. Herbivorous suspension-feeding bivalves have low proteolytic capacities. Carnivorous septibranchs have high proteolytic capacities. Deposit-feeding bivalves range from low to intermediate proteolytic capacities. The adaptive significance of the latter case is not clear.
3. Gastric volume, gastric pH, gastric protease secretion and digestive diverticular proteolytic activity appear to vary in relation to tidal events. It is not known if these are exogenous or endogenous rhythms.
4. The evolution of protein digestion in bivalves has been biochemically conservative. Proteases found in bivalves occur universally in the animal kingdom. Since quantitative changes are relatively simple evolutionary events, and since nothing is known of the primary structure of bivalve proteases, proteases and the process of protein digestion are poor indicators of phylogenetic relationships.

Literature Cited

Avise, John C.
1974. Systematic value of electrophoretic data. Syst. Zool. 23: 465 - 481 (September 1974)

Ballentine, Dorthy & John Edward Morton
1956. Filtering, feeding, and digestion in the lamellibranch *Lasaea rubra.* Journ. mar. Biol. Assoc. U. K. 35: 241 - 274
Bernard, Frank R.
1974. Septibranchs of the Eastern Pacific (Bivalvia Anomalodesmata). Allan Hancock Monogr. Mar. Biol. 8: 1 - 279 (28 February 1974)
Knudsen, Jøroen
1970. The systematics and biology of abyssal and hadal Bivalvia. Galathea Rprt. 11: 241 pp.; 20 plts.; 132 text figs. (6 Nov. 1970)
Koehn, Richard K. & J. B. Mitton
1972. Population genetics of marine pelecypoda. I. Ecological heterogeneity and evolutionary strategy at an enzyme locus. Amer. Natur. 106: 47 - 56
Kozlovsakay, E. P. & V. E. Vaskovsky
1970. A comparative study of proteinases of marine invertebrates. Comp. Biochem. Physiol. 34: 137 - 142 (15 January 1970)
Lanoton, Richard W. & P. A. Gabbott
1974. The tidal rhythm of extracellular digestion, and the response to feeding in *Ostrea edulis.* Marine Biol. 24: 181 - 187
Levinton, Jeffrey S.
1975. Levels of genetic polymorphism at two enzyme encoding loci in eight species of the genus *Macoma* (Mollusca: Bivalvia). Marine Biol. 33: 41 - 47
Levinton, Jeffrey S. & Doreen L. Fundiller
1975. An ecological and physiological approach to the study of biochemical polymorphisms. Proc. 9th Europ. mar. biol. Symp. pp. 165 - 178
Mansour, K.
1946. Food and digestive processes of the lamellibranchs. Nature 157: 482 (13 April 1946)
Mansour, K. & F. G. Zaki
1946. The digestive diverticula of *Unio prasidens* as organs of secretion. Proc. Egypt. Acad. Sci. 2: 38 - 44
Mansour-Bek, J. J.
1946. The digestive enzymes of *Tridacna elongata* Lam. and *Pinctada vulgaris* L. Proc. Egypt. Acad. Sci. 1: 13 - 20
Mathers, Nigel F.
1974. Digestion and pH variation in two species of oysters. Proc. Malacol. Soc. London 41: 37 - 40
Morton, Brian S.
1970. The tidal rhythm and rhythm of feeding and digestion in *Cardium edule.* Journ. mar. Biol. Assoc. U. K. 50: 499 - 512
1973. A new theory of feeding and digestion in the filter-feeding Lamellibranchia. Malacologia 14: 63 - 79 (23 January 1973)
Morton, John Edward
1956. The tidal rhythm and action of the digestive system of the lamellibranch *Lasaea rubra.* Journ mar. Biol. Assoc. U. K. 35: 583 - 586
Owen, Gareth
1956. Observations on the stomach and digestive diverticula of the Lamellibranchia. II. The Nuculidae. Quart. Journ. micr. Sci. 97: 541 - 567
1974. Feeding and digestion in the Bivalvia. Adv. Comp. Physiol. Biochem. 5: 1 - 35
Pelseneer, Paul
1891. Contribution à l'étude des lamellibranches. Arch. Biol. 11: 147 - 312 (August 1891)
Pohlo, Ross H.
1969. Confusion concerning deposit-feeding in the Tellinacea. Proc. malacol. Soc. London 38: 361 - 364
Purchon, Richard Denison
1956. The stomach in the Protobranchia and Septibranchia (Lamellibranchia). Proc. Zool. Soc. London 127: 511 - 525 (Dec. 1956)
1957. The stomach in the Filibranchia and Pseudolamellibranchia. Proc. Zool. Soc. London 129: 27 - 60 (September 1957)
1958. The stomach in the Eulamellibranchia; Stomach Type IV. Proc. Zool. Soc. London 135: 431 - 489 (December 1958)
1960. The stomach in the Eulamellibranchia; stomach types IV and V. Proc. Zool. Soc. London 135 (3): 431 - 489; 19 text figs. (20 October 1960)
1963. Phylogenetic classification of the Bivalvia, with special regard to the Septibranchia. Proc. malacol. Soc. London 35: 71 - 80
1971. Digestion in the filter-feeding bivalves — a new concept. Proc. malacol. Soc. London 39: 253 - 262
Reid, Robert G. B.
1966. Digestive tract enzymes in the bivalves *Lima hians* Gmelin, and *Mya arenaria* L. Comp. Biochem. Physiol. 17: 417 - 433
1968. The distribution of digestive tract enzymes in lamellibranchiate bivalves. Comp. Biochem. Physiol. 24:727 - 744 (15 Sept. 1968)
1971. Criteria for categorizing feeding types in bivalves. The Veliger 13 (4): 358 - 359 (1 April 1971)
1977. Gastric protein digestion in the carnivorous septibranch *Cardiomya planetica* Dall; with comparative notes on deposit and suspension-feeding bivalves. Comp. Biochem. Physiol. 56A: 573 - 575 (1 January 1977)
Reid, Robert G. B. & Kathy Rauchert
1970. Proteolytic enzymes in the bivalve mollusc *Chlamys hericius* Gould. Comp. Biochem. Physiol 35: 689 - 695 (15 July 1970)
1972. Protein digestion in members of the genus *Macoma* (Mollusca: Bivalvia). Comp. Biochem. Physiol. 41A: 887 - 895 (1 Dec. 1972)
1976. Catheptic endopeptidases and protein digestion in the horse clam *Tresus capax* (Gould). Comp. Biochem. Physiol. 54B: 467 to 472 (15 December 1976)
Reid, Robert G. B. & Alison Reid
1969. Feeding processes in members of the genus *Macoma* (Mollusca: Bivalvia). Canad. Journ. Zool. 47: 649 - 657. (November 1969)
1974. The carnivorous habit of members of the septibranch genus *Cuspidaria* (Mollusca: Bivalvia). Sarsia 56: 47 - 56 (8 July 1974)
Rosén, Birger
1949. Proteases in the digestive gland of lamellibranchs. Archiv Demi. 1: 205 - 211
Strickland, John D. H.
1965. Production of organic matter in the primary steps of the marine food chain. *In*: Chemical Oceanography 1: 477 - 610. Acad. Press, London, New York
Yonge, Charles Maurice
1928. Structure and function of the organs of feeding and digestion in the septibranchs, *Cuspidaria* and *Poromya.* Phil. Trans. Roy. Soc. Ser. B. 26: 221 - 263 (30 June 1927)
1930. The crystalline style of the Mollusca and a carnivorous habit cannot normally co-exist. Nature 125 (3151): 444 - 445 (22 March 1930)
1937. Evolution and adaptation in the digestive system of the Metazoa. Biol. Revs. Cambridge Phil. Soc. 12: 87 - 115